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Robin M. Overstreet

Gulf Coast Research Laboratory, robin.overstreet@usm.edu

Stephen S. Curran

Gulf Coast Research Laboratory

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Defeating diplostomoid dangers in USA catfish aquaculture

Robin M. Overstreet and Stephen S. Curran

Department of Coastal Sciences, The University of Southern Mississippi, P.O. Box 7000, Ocean Springs, Mississippi 39566-7000, USA

Key words: Diplostomoidea, *Clinostomum*, aquaculture, pathology, catfish, birds, snails, control methods, USA

Abstract. Diplostomoid digenean metacercariae have caused widescale mortalities of channel catfish, *Ictalurus punctatus* (Rafinesque), at aquaculture farms in Louisiana, Mississippi and Arkansas, USA. Originally, based on a tentative diagnosis, the industry considered the primary harmful agent to be an introduced species from Europe, *Bolbophorus confusus* (Krause, 1914), frequently reported from the American white pelican, *Pelecanus erythrorhynchos* Gmelin. Our group has now shown, using ITS 1-2 plus three more-conservative gene fragments, that two sympatric species of *Bolbophorus* exist in the American white pelican. One, *B. damnificus* Overstreet et Curran, 2002, infects the musculature of catfish, and the other, probably not *B. confusus*, does not infect catfish. However, at least four other pathogenic diplostomoids and a clinostomoid infect the catfish, and they use at least four different snail hosts, including the planorbids *Planorbella trivolvis* (Say) and *Gyraulus parvus* (Say), the physid *Physella gyrina* (Say) and a lymnaeid. Two metacercariae, *B. damnificus* and *Bursacetabulus pelecus* Dronen, Tehrany et Wardle, 1999, infect the catfish and mature in the pelican; two others, *Austrodiplostomum compactum* (Lutz, 1928) and *Hysteromorpha* cf. *triloba* (Rudolphi, 1819), mature in cormorants; one, *Diplostomum* sp., matures in seagulls and at least one, *Clinostomum marginatum* (Rudolphi, 1819), matures in herons, egrets and other wading birds. Consequently, management of catfish ponds relative to digenean infections requires considerable biological information on the fish, bird, and snail hosts as well as the parasites.

Catfish farms in the Southeast United States of America, especially Louisiana and Mississippi, had recent heavy losses of the channel catfish, *Ictalurus punctatus* (Rafinesque), from trematode infections. The actual amount of losses is not clear because many large affected farms completely stopped producing catfish. The number of reported outbreaks seems to be less now; however, many farmers had and still have no understanding about the infections. In 2002, only 15% of the foodsize catfish producers reported familiarity with the problem, and about 45% still were not aware of it (APHIS Veterinary Services Info Sheet, November 2003, no. N408.1103). The loss of catfish to infections started during the mid 1990s in Louisiana and was brought to our attention by then catfish-farm manager, Rusty Gaudé. Initially, we were under the assumption from catfish farmers and state agents that a single digenean was responsible, even though Gaudé brought us one species of snail shedding two species of cercariae. The cercaria of one species killed catfish fingerlings within a few minutes when a moderate number was allowed to penetrate the fish. The other cercaria was a species of “yellow grub”, *Clinostomum* sp. Morphological features of the cercaria that killed the fish appeared very similar to that identified as *Bolbophorus confusus* (Krause, 1914) by Fox (1965a), and the metacercaria, located under and within the dermis of the fish from the ponds, also appeared superficially as *B. confusus*. In 1999–2000, Terhune et al. (2002) sampled 32

farms in Mississippi and reported at least 32% of 821 ponds positive for *B. confusus*. Many farmers thought that ridding ponds of the reported host for *B. confusus*, the American white pelican, would solve the problem. The problem got more complicated when our initial genetic sequence data from adults in the intestine of the pelican and from metacercariae in the catfish did not match. As Gaudé and others provided additional snails for our investigation, we encountered additional digenean species. This article reports our findings from those investigations, the actual and potential agents responsible for catfish mortalities, the complexities involved with those agents and a discussion on how to manage ponds.

MATERIALS AND METHODS

Catfish were made available from ponds, mostly near Catahoula in St. Martin Parish and Wisner in Franklin Parish, Louisiana, by farm managers and agents of the Louisiana State University, Louisiana Cooperative Extension Service. These catfish included infected adults and fingerlings with metacercariae necessary for morphological, experimental, and molecular studies as well as uninfected fingerlings from ponds with uninfected snails for exposing to various cercariae from infected snails. Infected snails were obtained from ponds known to have infected catfish, and some were already screened for infections when we received them. Exposure of

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fingerlings to cercariae from different snail species was conducted by introducing fingerlings into a 20-cm Carolina culture dish a third filled with 500 ml of either filtered pond water or aged well water at room temperature (approximately 25°C) containing either cercariae from a single snail or a shedding infected snail. In additional cases, some with exposure to *Bolbophorus damnificus* Overstreet et Curran, 2002 and others with exposure to a clinostome, the catfish, *Gambusia affinis* (Baird et Girard) and *Poecilia reticulata* Peters were placed with a very large number of cercariae from several snails in 19-l buckets about a quarter filled. The exact number of cercariae in no case was counted, but, typically, fingerlings were left with cercariae for different lengths of time. Cercariae and resulting metacercariae were examined alive with and without neutral red. Representative metacercariae were both heat-killed and cold-killed, fixed in 5% phosphate buffered formalin, stained in Van Cleave's haematoxylin or Semichon's acetocarmine and mounted in Canada balsam. Methods for experimental infections in actual and potential definitive hosts were described by Overstreet et al. (2002). Adult material was collected from various birds in St. Martin Parish as well as elsewhere in coastal and inland areas of Louisiana and Mississippi. Representative metacercariae and adults also were fixed in Bouin's solution or 10% buffered formalin for histological sections at 4 µm. In some cases, representative metacercariae were placed in a SED buffer (saturated NaCl; 250 mM ethylene diamine tetraacetic acid pH 7.5; 20% dimethyl sulfoxide) or 95% ethanol, and their DNA was extracted and sequenced as described by Overstreet et al. (2002). Common names for hosts and experimental animals, the only names that will be recognised by some of the readership, occur in Table 1.

Table 1. Common names of actual or potential strigeid fish-, frog-, avian- and snail-hosts from the United States mentioned in the text.

Common name	Scientific name
channel catfish	<i>Ictalurus punctatus</i> (Rafinesque)
blue catfish	<i>Ictalurus furcatus</i> (Valenciennes)
bluegill	<i>Lepomis macrochirus</i> Rafinesque
western mosquitofish	<i>Gambusia affinis</i> (Baird et Girard)
guppy	<i>Poecilia reticulata</i> Peters
green swordtail	<i>Xiphophorus helleri</i> Heckel
rainbow trout	<i>Oncorhynchus mykiss</i> (Walbaum)
mottled sculpin	<i>Cottus bairdi</i> Girard
grass carp	<i>Ctenopharyngodon idella</i> Valenciennes
black carp	<i>Mylopharyngodon piceus</i> (Richardson)
southern leopard frog	<i>Rana sphenoccephala</i> Cope
American white pelican	<i>Pelecanus erythrorhynchos</i> Gmelin
brown pelican	<i>Pelecanus occidentalis</i> Linnaeus
double-crested cormorant	<i>Phalacrocorax auritus</i> (Lesson)
Neotropic cormorant	<i>Phalacrocorax brasilianus</i> (Gmelin)
great blue heron	<i>Ardea herodias</i> Linnaeus
great egret	<i>Ardea alba</i> Linnaeus
snowy egret	<i>Egretta thula</i> (Molina)
black-crowned night-heron	<i>Nycticorax nycticorax</i> (Linnaeus)
marsh rams-horn	<i>Planorbella trivolvis</i> (Say)
ash gyro	<i>Gyraulus parvus</i> (Say)
tadpole physa	<i>Physella gyrina</i> (Say)

RESULTS

Digenean species

All members of the superfamily Diplostomoidea Poirier, 1886 that we encountered in the pond-raised channel catfish from Louisiana and Mississippi and in experimental fish matured in the intestine of an avian host. We observed a total of five of these diplostomoid species, but a related strigeid member of the superfamily Clinostomoidea Lühe, 1901 also infected the catfish as well as the buccal cavity and oesophagus of its bird hosts. Additional digenean species that do not infect catfish also infected snails from a few of the ponds. The metacercaria of each species differed in appearance when fixed with or without heat, making comparisons with the literature difficult.

Two of the diplostomoids infected the American white pelican, *Pelecanus erythrorhynchos* Gmelin (Fig. 1). The first, *Bolbophorus damnificus*, produced a metacercaria termed a "prodiplostomulum", a metacercaria that has pseudosuckers and encysts in its fish intermediate host (Shoop 1989). In both experimental and natural infections, the prodiplostomulum usually encysted immediately under the dermis in the caudal and dorsal regions of the catfish but occasionally deeper in the muscle, in fins, in muscles of the head and inside the skull. The cercaria developed in the planorbid *Planorbella trivolvis* (Say). The complete life cycle for *B. damnificus* based on our observations and those of Fox and Olson (1965) represents the general pattern for all the species included herein. Worm eggs deposited in water from the pelican's faeces hatch after about two weeks. After penetrating into *P. trivolvis*, the miracidium produced a mother sporocyst in the mantle that produced a large number of daughter sporocysts, which replaced most of the snail's hepatopancreas, which in turn produced large numbers of furcocercous cercariae. These cercariae developed within about 30 days after the single miracidium entered the snail, and, after emerging, each penetrated the catfish, lost its tail, located near the dermis or elsewhere, encysted and developed as a metacercaria and became infective to pelicans after about 28 days. Once the catfish was eaten by, or experimentally fed to, the pelican, it started producing eggs within less than 72 h. No experimentally fed metacercaria fed to or placed within a variety of experimental hosts other than adult American white pelicans (*Pelecanus erythrorhynchos* Gmelin) developed to maturity (e.g., Overstreet et al. 2002). The second diplostomoid that matured in the white pelican was *Bursacetabulus pelecaneus* Dronen, Tehrany et Wardle, 1999. It produced a "diplostomulum", a metacercaria with pseudosuckers that does not encyst in the fish host (Shoop 1989). The metacercaria developed in the brain, spinal cord, optic nerve and eye of the catfish after emerging from the planorbid *Gyraulus parvus* (Say).

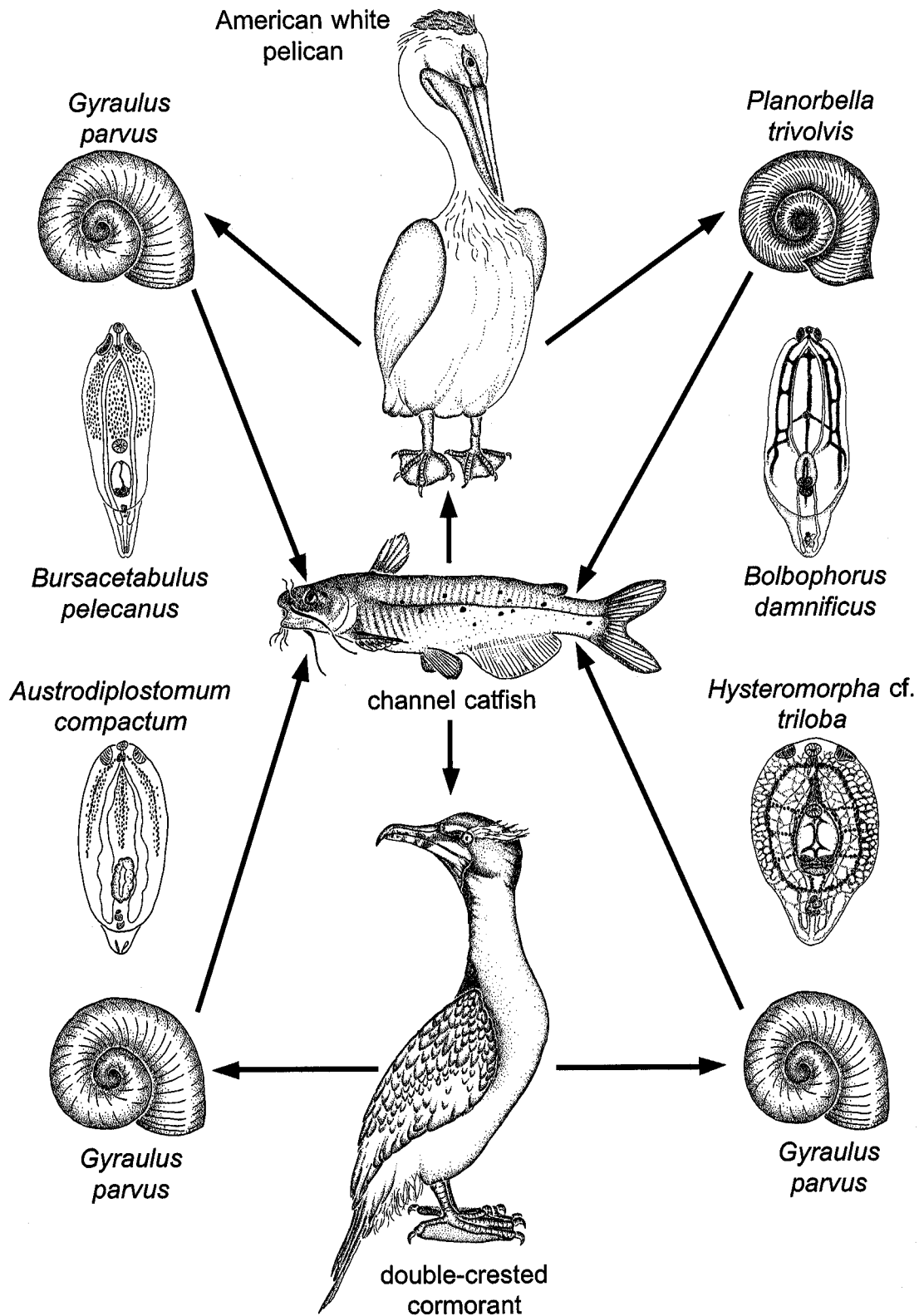


Fig. 1. Life cycles of four digeneans from the channel catfish (*Ictalurus punctatus*) that use pelicans, primarily the American white pelican (*Pelecanus erythrorhynchos*), and cormorants, primarily the double-crested cormorant (*Phalacrocorax auritus*), as definitive hosts on southeastern USA catfish farms.

Another two diplostomoid species infected cormorants (Fig. 1). Two cormorants, *Phalacrocorax auritus* (Lesson) and *Phalacrocorax brasilianus* (Gmelin), both contained the two digeneans and fed in the catfish ponds. The first digenean, *Austrodiplostomum compactum* (Lutz, 1928), had a diplostomulum metacercaria that produced experimental infections in the brain, optic nerve and eye, but, in natural infections, we observed the metacercariae primarily free in the vitreous humour of the eye. The cercaria developed in *G. parvus*. The second digenean, *Hysteromorpha* cf. *triloba* (Rudolphi, 1819), had a diplostomulum metacercaria that was either free or encapsulated by host fibrotic material, superficially appearing like a pliable cyst. It was usually deep in the musculature and often associated with the vertebral column. The cercaria also developed in the snail *G. parvus*.

Other birds also hosted a diplostomoid that infected the catfish. Seagulls, probably several species, were infected with a diplostomulum of *Diplostomum* sp. (a species in the *D. spathaceum* [Rudolphi, 1819] complex) that infected the lens of the catfish eye. What we considered to be the cercaria was shed by an unidentified lymnaeid pulmonate snail.

Hérons, including at least *Ardea herodias* Linnaeus and *Nycticorax nycticorax* (Linnaeus), and egrets, at least *Ardea alba* Linnaeus and *Egretta thula* (Molina), fed on catfish and hosted at least two species of the related strigeid genus *Clinostomum* Leidy, 1856. Those clinostome species included the species *Clinostomum marginatum* (Rudolphi, 1819), which occurred free or encapsulated, but not encysted (Larson et al. 1988), under the dermis of catfish.

Pathogenic responses including mortality

Cercariae of all the indicated species can produce mortality in the catfish, if it serves as an acceptable second intermediate host. The time of death after exposure depended on the species of digenean, virulence of the individual cercaria, the number of cercariae entering the host, size of fish, tissues and organs infected, associated bacterial complications, environmental conditions and presumably a few other biotic and abiotic factors.

Bolbophorus damnificus seemed especially pathogenic to the catfish compared with the other species (Figs. 2–16). Infections of fingerlings could produce death within 5 min when infected by a few hundred individuals of the cercaria. Exposure to fewer cercariae proportionally lengthened the time between cercarial penetration and death. When the time fingerlings remained in a bucket with thousands of cercariae was reduced to 2 min, some fish with about 50–100 established metacercariae survived at least 1–2 weeks (Figs. 8, 9). In some of those cases, severe hydropic degeneration and necrosis were evident in the kidney of infected but not uninfected fish; in some cases, these pathological alterations were associated with mortality,

and, in others, they were not. In chronic infections, the host often responded with a thick fibrotic encapsulation around the cyst, depending on the site and other features of infection (Figs. 2–7). Haemorrhaging was often associated with these infections, and, when the metacercaria occurred just below the dermis, blood was visible grossly (Figs. 2, 3, 5, 10). Occasionally, haemorrhaging associated with metacercariae located deep within the musculature was also obvious (Fig. 7). In 10-cm total length fingerlings from a Mississippi pond, all had 48 or fewer metacercariae. Larger fingerlings in Louisiana had a somewhat higher maximal number, and adults could have over a hundred. We seldom saw infections that high.

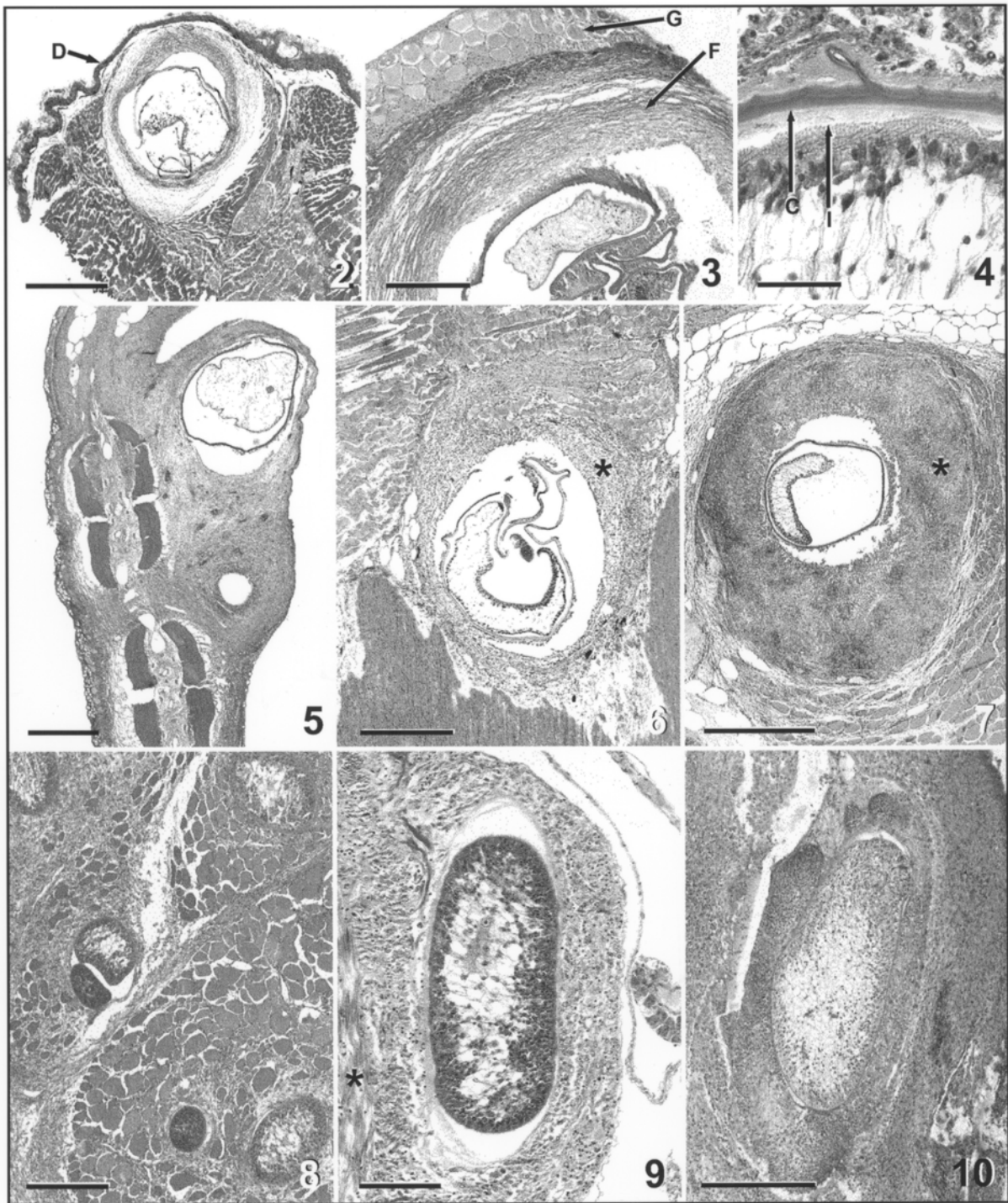
Our objective was not to determine exactly how many cercariae under what conditions would produce mortality but rather to establish infections. A fingerling catfish apparently withstood a much higher number of penetrating cercariae of *Bursacetabulus pelecus* than of *Bolbophorus damnificus* because a host could survive an infection with a larger number of developing metacercariae. Two or three weeks after exposure, we have seen several hundred migrating metacercariae of *B. pelecus* in the brain, spinal cord, optic nerve and eye in relatively healthy appearing individuals. Adult catfish could withstand an eyeful of *A. compactum* (Fig. 14), even though the retina was often so affected that the fish was probably blind. Blindness can also affect fishes other than the catfish (Fig. 15). We found no specimen of *Bolbophorus* sp. in catfish, but *Gambusia affinis* and *Poecilia reticulata* were highly susceptible (Figs. 11–13) and died soon after exposure to large number of the cercariae. *Gambusia affinis* could also handle large numbers of *C. attenuatum*, at least for up to 39 days following initial infection (e.g., Fig. 16).

DISCUSSION

We report several important findings above that relate to catfish farming and management of diplostomoid digenean infections. First, more than one species of digenean has been responsible for the mass mortalities in the Southeast USA even though some species are more abundant or more pathogenic in catfish than in other fishes. Second, more than one species of bird harbours the infectious pathogens, making control of the avian hosts a difficult, labour-intensive and nearly impossible means to eliminate or significantly reduce infections in catfish ponds. Third, more than one species of snail hosts these and other parasites, but control of the snails seems to be the method most acceptable for regulating agencies as well as the easiest and cheapest means of managing infections.

Digeneans and their hosts

Scientific and popular articles in aquaculture and commercial catfish literature led farmers, managers and



Figs. 2–10. *Bolbophorus damnificus* in *Ictalurus punctatus*, the channel catfish. Haematoxylin and eosin stained paraffin sections. **Fig. 2.** Well-developed prodiplostomum in skeletal muscle beginning to protrude through dermis (D) of caudal area and exhibiting loose fibrotic encapsulation and grossly visible haemorrhaging. **Fig. 3.** Another haemorrhaging peripheral cyst with hyperplastic layers of goblet cells (G) and thick fibrotic layer (F). **Fig. 4.** Close up of Fig. 7 showing worm integument (I) and cyst wall. In addition to the differentiated “homogeneous” internal (C) and external layers of the cyst wall of parasite and host origin, respectively, a portion of a thick, extensive encapsulation of host inflammatory cells is apparent on the top of the figure. **Fig. 5.** Peripheral cyst located on caudal fin, with conspicuous fibrotic encapsulations of that and adjacent cyst out of the plane of the section. **Fig. 6.** Deeply embedded cyst within muscles exhibiting moderate, loose host inflammatory encapsulation (*). The cyst appeared heavily inflamed in the living fish. **Fig. 7.** Similarly embedded cyst exhibiting extensive, dense inflammatory encapsulation (*) with surrounding fibrous layers and fatty deposition. **Fig. 8.** A one-week experimental infection showing five metacercariae in mandibular muscles prior to cyst-formation with low grade spreading inflammatory response. **Fig. 9.** A developing metacercaria in serosal layer of oesophagus (* = circular muscle layer) demonstrating moderate relatively confined inflammatory response from the same experimental infection as in Fig. 8. **Fig. 10.** A natural infection showing a more developed metacercaria than in Fig. 9 within dermis demonstrating extensive haemorrhaging. Scale bars: Figs. 2, 6 = 250 μ m; Figs. 3, 10 = 200 μ m; Fig. 4 = 30 μ m; Fig. 5 = 300 μ m; Fig. 7 = 425 μ m; Fig. 8 = 150 μ m; Fig. 9 = 80 μ m.

others to think that only a single digenean, usually identified as *Bolbophorus confusus*, was responsible for all digenean-caused mortalities (e.g., Venable et al. 2000, Terhune et al. 2002). Whereas that organism, *Bolbophorus damnificus*, was the most visible one and probably the most responsible one in many cases, it probably was and still is joined by a few others. When considering a single cause, however, associated parties assumed that a single bird was responsible for transmitting infections.

Knowing the species of parasite infecting the catfish allows proper management since each digenean has a different life history. *Bolbophorus damnificus* occurs in the American white pelican in Louisiana and Mississippi but also northerly into North Dakota, Saskatchewan, Manitoba and elsewhere along the migratory Mississippi flyway route from the Southeast USA (Overstreet et al. 2002). It was additionally reported from catfish in California (Levy et al. 2002) where a different population of *Pelecanus erythrorhynchos* occurs. We have seen infections in *Pelecanus occidentalis* Linnaeus, but that pelican rarely leaves the coastal region to where it could feed on the cultured catfish. Infections of *B. damnificus* also occur in wild channel catfish, sometimes in relatively high numbers, and it presumably occurs in other catfishes and bullheads, but the specific identification requires confirmation. As an example of heavy infections in wild channel catfish, Andrew Mitchell (USDA, Agriculture Research Service [ARS], pers. comm.) noted up to 50 metacercariae in 2 cm of the caudal portion of fish in Lake Chico, Arkansas, where habitat conditions are similar to those in ponds.

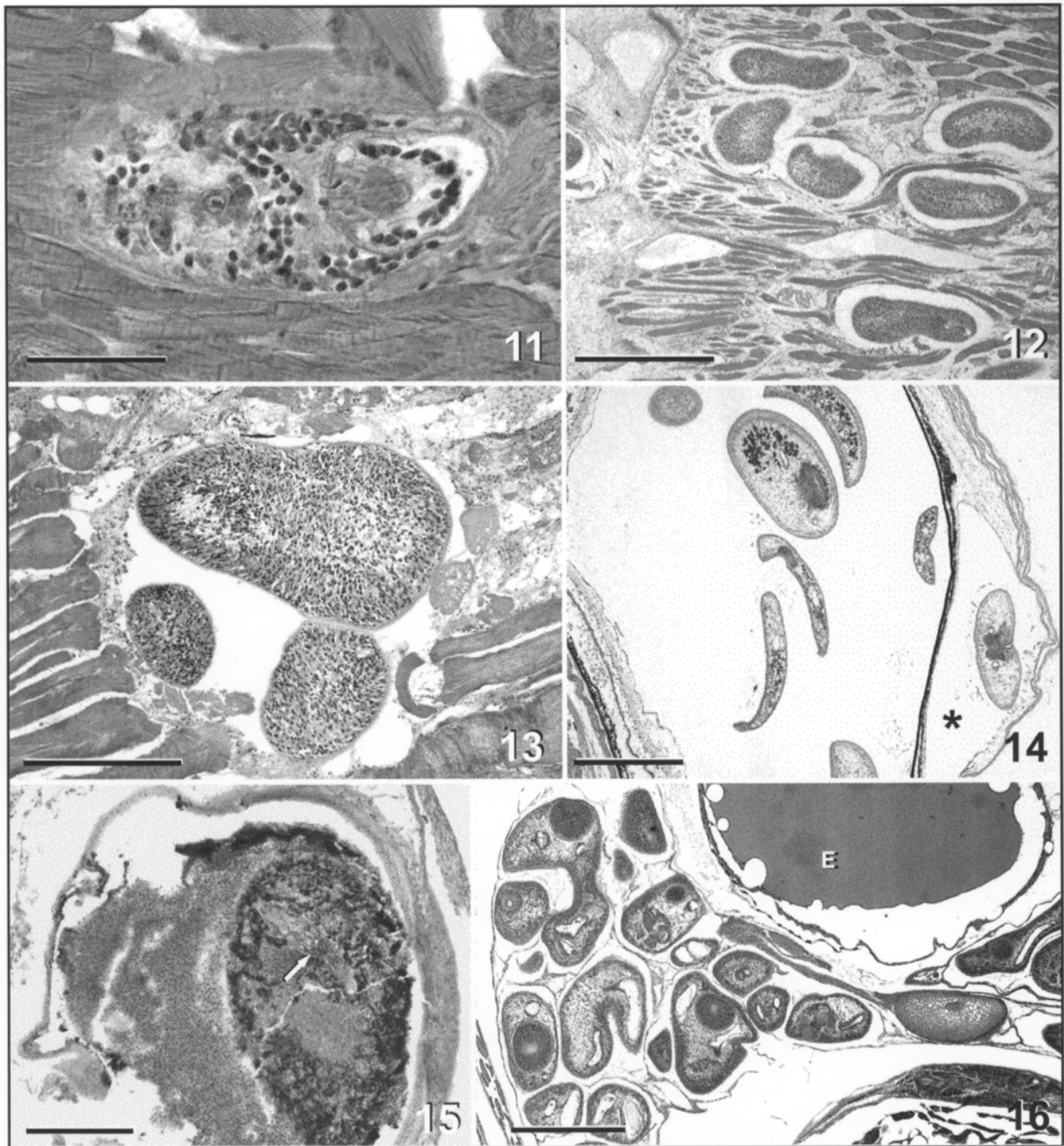
As indicated in the introduction, confusion about the identification of *Bolbophorus confusus* has existed in the past. Molecular data prompted an investigation of what was originally considered to be *B. confusus* in Louisiana and Mississippi. Overstreet et al. (2002) showed, using morphological features as well as COI, ITS (ITS-1, 5.8S rRNA and ITS-2), 18S rRNA and 28S rRNA genetic fragments, that the American white pelican harboured concurrent infections of *B. damnificus* and a morphologically very similar species, *Bolbophorus* sp. of Overstreet et al. (2002). Later, Levy et al. (2002), using an 18S rRNA amplicon, also reported specimens that we consider conspecific with *Bolbophorus* sp. of Overstreet et al. (2002). Based on the corresponding 795-base pair fragment sent to GenBank from both studies, we determined using a LI-COR AlignIR Alignment Report that there was a 100.0% similarity with what the two studies consider *Bolbophorus* sp., a 99.8% similarity in *B. damnificus* and 97.0% similarity between the two. Overstreet et al. (2002) also separated those two species with other amplicons. As discussed by Overstreet et al. (2002), we doubt that *Bolbophorus* sp. is conspecific with *B. confusus* or that *B. confusus* (sensu stricto) from Europe occurs in North America. We are presently investigating

that problem. Only *B. damnificus* was shown to infect the catfish by feeding experiments, cercarial exposure and molecular data (Levy et al. 2002, Overstreet et al. 2002).

Fox and Olson (1965) conducted life-cycle research of what they considered at the time to be *B. confusus* in Montana. They demonstrated that temperature dictated developmental times of most stages, with approximately 25°C being optimal or the best for temperatures tested. Fox (1965a) was not able to infect snail species of *Gyraulus* Charpentier, *Physa* Draparnaud or *Lymnaea* Lamarck, but he and Olson (Fox 1965a, Fox and Olson 1965, Olson 1966) did experimentally infect a variety of fishes in the families Salmonidae, Cyprinidae, Catostomidae, Ictaluridae, Poeciliidae, Centrarchidae, and Cottidae. Olson (1966) and Fox and Olson (1965) considered the channel catfish (and *Cottus bairdi* Girard) an abnormal host because the produced metacercariae were small and poorly developed. Overstreet et al. (2002) determined that the morphologically very similar *B. damnificus* and *Bolbophorus* sp. of Overstreet et al. (2002) both occurred near the area where Fox and Olson conducted studies. Whether Fox and Olson (1965) conducted all or any of their exposures with *B. damnificus* is questionable because we experimentally infected two poeciliids, *Gambusia affinis* and *Poecilia reticulata*, with *Bolbophorus* sp. of Overstreet et al. (2002) (Figs. 11–13); also, Levy et al. (2002) experimentally infected the poeciliids *P. reticulata* and *Xiphophorus helleri* Heckel as well as the bass hybrid (*Morone saxatilis* [Walbaum] × *Morone chrysops* [Rafinesque]) with that digenean. We could not infect *G. affinis* with *B. damnificus*, but Levy et al. (2002) did recover a single individual in addition to three of *Bolbophorus* sp. in a single specimen of *P. reticulata*. Fox and Olson (1965) obtained adult specimens, which were not described well enough to identify, by feeding infected *G. affinis* to *Pelecanus erythrorhynchos*. Nevertheless, based on our observations, we expect that Fox and Olson conducted most or all their experimental work with *Bolbophorus* sp. of Overstreet et al. (2002) (reported as *B. confusus*) and that the general development of both confirmed North American species of *Bolbophorus* Dubois, 1935, *B. damnificus* and *Bolbophorus* sp., is very similar.

The other catfish diplostomoid maturing in pelicans, *Bursacetabulus pelecatus*, to date has been seen in both *P. erythrorhynchos* and *P. occidentalis* in Mississippi and Louisiana in addition to the reports from Texas (Dronen et al. 2003). Molecular work to show relationships among this and some of the other diplostomes will be reported by Charles Blend, presently at Texas A & M University, and colleagues (pers. comm.).

The first of the two cormorant-infecting digeneans, *Austrodiplostomum compactum*, was originally described from *Phalacrocorax brasilianus* in Venezuela (Lutz 1928) and has been reported from the same bird,



Figs. 11–16. Strigeids in haematoxylin and eosin stained paraffin sections. **Fig. 11.** Cercaria of *Bolbophorus* sp. from *Planorbella trivolvis* in skeletal muscle of experimentally infected guppy at 18 h. **Fig. 12.** Nine-day-old metacercariae of *Bolbophorus* sp. in caudal region of experimentally infected *Gambusia affinis* exhibiting haemorrhaging. **Fig. 13.** Close-up of metacercariae from same infection as in Fig. 12 showing muscle necrosis and little cellular inflammatory infiltrate. We did not confirm an infection of this species in *Ictalurus punctatus*. **Fig. 14.** Section through eye of naturally infected *I. punctatus* showing a few of the numerous diplostomula of *Austrodiplostomum compactum* in the vitreous humour. In some cases the retina is hypertrophied, distorted, disrupted or otherwise severely damaged and in others it appears normal. The single specimen in the anterior chamber (*) to the right of the iris may be *Bursacetabulus pelecyanus*. **Fig. 15.** Eye of *G. affinis* experimentally infected with cercariae of *A. compactum* after losing tail, located in eye showing totally destroyed retina and extensive haemorrhaging. **Fig. 16.** Metacercariae of *Clinostomum attenuatum* at 39 days in *G. affinis* infected with cercariae from *P. trivolvis*. Note several specimens in the ovary between two eggs (E); the entire ovary was heavily infected as were other tissues within the body cavity. This digenean did not infect the channel catfish. Scale bars: Fig. 11 = 45 µm; Fig. 12 = 500 µm; Fig. 13 = 250 µm; Fig. 14 = 400 µm; Fig. 15 = 125 µm; Fig. 16 = 350 µm.

Ictalurus furcatus (Valenciennes) (reported as *I. meridionalis* [Günther], a junior synonym) and the planorbid snail *Biomphalaria obstructa* (Morelet) in the State of Tabasco, Mexico, by Pineda-López (1985). Because of the wide-ranging migration of the host bird, we do not question the parasite identification. We also collected it more frequently from *Phalacrocorax auritus*. The digenean was considered by Dubois (1970) to be a junior synonym of *A. mordax* Szidat et Nani, 1951, originally described from *P. brasiliensis* and the atherinid (silver-side) *Odontesthes bonariensis* (Valenciennes) (reported as the synonym *Basilichthys bonariensis*) in Argentina. Even though the synonym of the digenean was accepted by others, Kohn et al. (1995), who reported the metacercaria from a sciaenid fish, restated their earlier opinion accepting both species. Specimens in the vitreous humour in fig. 33 of Hoffman (1979) appear to us as *A. compactum*, with *Diplostomum* as a misleading identification.

Our identification of *Hysteromorpha* cf. *triloba* is uncertain, even though *H. triloba* has been reported from North America numerous times (e.g., Chandler and Rausch 1948, Huggins 1954), but its adult has been confirmed from only cormorant final hosts. *Hysteromorpha triloba* was originally described from Europe, and we suspect the American material represents a separate species (probably *Hysteromorpha corti* [Hughes, 1929]) from that in Europe and are investigating material molecularly to determine if the slight morphological differences constitute specific differences. In North America, bullheads, catfishes, cyprinids and catostomids have been listed as second intermediate hosts (Huggins 1954, Hoffman 1999). Huggins (1954) was unable to experimentally infect gulls or day-old chicks or ducklings.

Seagulls are hosts to several species of *Diplostomum* von Nordmann, 1832 (e.g., Dubois 1970). We are in the process of investigating the identity of the species in the *D. spathaceum* (Rudolphi, 1819) complex that infected the eye lens of the catfish in Louisiana. It was reported as being hosted by pelicans (Hoffman 1979, MacMillan 1985), but we assume those reports actually refer to a species of *Bolbophorus* (see Overstreet et al. 2002).

We presently are also investigating what we consider to be at least two or possibly three “yellow grubs” in the Southeast USA. We have seen what we tentatively call *Clinostomum marginatum* from catfish in ponds, but not in the ponds from which we collected *Planorbella trivolvis* shedding clinostome cercaria. With exposure of thousands of these cercariae presumed to be *C. attenuatum* Cort, 1913, we were unable to infect *Ictalurus punctatus*, *Lepomis macrochirus* Rafinesque) or tadpole of *Rana sphenoccephala* Cope. However, we readily infected *G. affinis*, exposed to small and large numbers of these, but 39-day-old specimens were not mature enough to clearly confirm the specific identity of these

according to the morphological features used by Cort (1913) and others. Some authors consider *C. marginatum* a junior synonym of *C. complanatum* (Rudolphi, 1814). For example, Gibson (1996) and Hoffman (1999) treated reports of all clinostomes in North America as *C. complanatum*. In fact, Gibson (1996) discussed that some authors consider it the only species in the genus. On the other hand, Forrester and Spalding (2003) list four species from Florida. We are uncertain about the identity of the North American species morphologically identifiable as *C. marginatum* by Cort (1913), but we tentatively consider it as such and consider it different from *C. complanatum* and *C. attenuatum*.

Posthodiplostomum centrarchi Hoffman, 1958, common in centrarchid fishes, was not observed in any catfish species. Some researchers (e.g., Dubois 1970) consider that species a subspecies of *P. minimum* (MacCallum, 1921), and *P. minimum* has been reported but not confirmed from wild channel catfish (Williams and Dyer 1992). At least *P. centrarchi* is present in centrarchids co-occurring in catfish ponds and has a third type of metacercaria termed a “neascus” that encysts but does not possess pseudosuckers. We determined that its cercaria developed in *Physella gyrina* (Say) from the ponds. Other digeneans such as plagiorchoids and echinostomes that do not infect the catfish also infect snails from the ponds.

Birds other than the pelican and cormorants that serve as strigeid hosts can prey on fingerling catfish in ponds, but they apparently do not consume as many catfish from the commercial stocks (Glahn et al. 1999). Over a half dozen of these birds harbour digeneans that transmit infections to catfish in Southeast ponds. Regardless of the number, keeping the birds away from the ponds would be advantageous to control predation on the fish. However, killing a large portion of these predators would not eliminate digenean infections and resulting mortalities. We have examined for digeneans in numerous avian species that occupy catfish ponds and that feed on both commercial catfish and native fishes that invade the ponds. In addition, we have examined the same and a few other piscivorous bird species in the general region that may serve as alternate definitive hosts of the parasites. Several of these host some of the parasites that could maintain infections by defecating in or near the ponds.

The Southeast ponds contained large numbers of at least members of Planorbidae (*Planorbella trivolvis* and *Gyraulus parvus*), Physidae (*Physella gyrina*) and Lymnaeidae. Those snails live primarily near the shoreline of the ponds, and that habitat promotes development of large numbers of them. Because of the ease in obtaining fish prey, the habitat attracts a large concentration of piscivorous birds capable of defecating into ponds and thereby infecting a relatively large percentage of those

snails with the entire series of digenean species. Since this shallow area is where most fingerling catfish inhabit and fingerlings are the most vulnerable stage in aquaculture to harm from penetration by cercariae and resulting infections, these young fish can and do become heavily infected and affected by a variety of digeneans.

Pathology

Large numbers of any digenean species that can infect *Ictalurus punctatus* can kill it, especially the young individuals. This is true even though the catfish can tolerate infections of the cercaria of some species more than others. Fingerlings seem to have a hard time accommodating 100 metacercariae of *Bolbophorus damnificus*, but they can tolerate, at least during the few weeks when observed, several times that number of the metacercariae of *Bursacetabulus pelecanus*. In fact, the Latin word “*damnificus*” refers to the harmful nature of that species to the fingerlings (Overstreet et al. 2002). Adult catfish in ponds occasionally contained well over a hundred specimens of *B. damnificus*, but they probably acquired them over time or when larger. The reason that only 48 or fewer individuals of *B. damnificus* were observed in Mississippi ponds could be because those with more had died. Fish infected with the much larger number of *B. pelecanus* presumably die after a few weeks as may those infected with fewer individuals in vulnerable sites in the nervous system or with bacterial infections or additional digenean species. We have observed bacteria associated with some of the lesions produced by the digeneans, and, under the appropriate conditions, some of these probably enhance disease conditions.

We know that cercariae of *Bolbophorus* sp. of Overstreet et al. (2002) did not infect the fingerling catfish tested. In our experiments exposing *Gambusia affinis* and *Poecilia reticulata*, we could kill specimens within minutes to days, depending on how long the fish remained in contact with large numbers of the cercaria. Levy et al. (2002) exposed *P. reticulata*, *Xiphophorus helleri*, hybrid bass and *I. punctatus* with a mixture of cercariae of *Bolbophorus* sp. and *B. damnificus*, and the exposure appeared to kill all of the first three hosts within 10 and 31 days with *Bolbophorus* sp. The authors, however, recovered a single specimen of *B. damnificus* as indicated above along with three of *Bolbophorus* sp. in one specimen of *P. reticulata* exposed to a sublethal infection with the two-species mixture of 10 cercariae. We never recognized a specimen of *B. damnificus* from exposures of either low or high numbers of the cercaria. Also, Fox (1965b), using what was probably *Bolbophorus* sp., determined that *Onco-rhynchus mykiss* (Walbaum) exposed to a sublethal dose of cercariae had a reduced haematocrit, reduced swimming ability and less ability to tolerate an increase in temperature from 21°C when compared with noninfected controls.

In some Mississippi ponds, fingerling catfish harboured many more metacercaria of *H. cf. triloba* than of *B. damnificus*, and presumably each fish has a threshold number of individuals it can tolerate relative to size of host and other factors. Szidat and Nani (1951) reported severe epizootics of *Austrodiplostomum mordax* in the brain of the atherinid *Odontesthes bonariensis*, the most susceptible of several hosts in Argentina. They considered infections capable of producing mortalities, but concurrent brain infections with the related *Tylodelphus destructor* Szidat et Nani, 1951 or sole infections with fewer specimens of *T. destructor* than of *A. mordax* were more pathogenic to the host. Of several strigeid digeneans in southeast Mexico, Pineda-López (1985) considered *Austrodiplostomum compactum* and *Clinostomum complanatum* as threats to aquaculture development because of their pathogenic nature. Lo et al. (1985) experimentally infected the loach *Misgurnus anguillicaudatus* (Cantor) (Cobitidae) in Taiwan and found that it took large numbers of *C. complanatum* in the dermis and other non-visceral tissues to produce malformation, lethargy, erratic swimming behaviour, immobility and death of the fish. In contrast, when the metacercaria in light infections was activated by administration of pesticides or changes in water temperature, it left the encapsulation, perhaps secreted a lytic enzyme, damaged host tissues and killed the host. We could infect *G. affinis* with a few hundred individuals of what we consider *Clinostomum attenuatum* in and among the viscera with little apparent distress, at least by day 39 of infection (Fig. 16).

Whereas one might expect a moderate infection of relatively small diplostomoid metacercariae in the eye lens to have an effect on the ability of a host to avoid predators, one might not consider such infections severe enough to kill individuals directly. That presumption is not true. Anne H. Larsen and Kurt Buchmann (Royal Vet. Agricult. Univ., Copenhagen, pers. comm.) determined this with *O. mykiss*, (5-month-old fry) experimentally exposed at 12°C with 10, 100, 600 and 1000 cercariae of *Diplostomum spathaceum* (sensu stricto). The cercariae were shed from *Radix peregra* (O.F. Müller) (= *Radix balthica* [Linnaeus]) collected in Lake Furesø near Copenhagen, Denmark. Approximately 60–80% of the experimental dose was recovered as metacercariae from the lens. Fish exposed to 600 and 1000 cercariae each died within 2 days, and those administered the lower dosages survived but some demonstrated altered behaviour. The research by Szidat and Nani (1951) and earlier studies demonstrated that large numbers of a related cercaria could produce death in experimental infections, especially in young individuals, which died rapidly from haemorrhaging of capillaries and obstructed blood vessels primarily in the head and brain. They report fish kills from the diplostome in wild, even though those cases were atypical ones where

cercariae could accumulate in large numbers. Hoffman (1979) indicated that the eye diplostomoid species considered as *D. spathaceum* may cause blindness in heavily infected catfish, a condition repeated by MacMillan (1985).

As indicated above, there is reason to assume that the cercariae of any diplostomoid species could produce death in susceptible catfish. The example of the fingerling's resistance to exposure of thousands of the clinostome cercariae demonstrated the difficulty in assessing infections. We know that the catfish can be infected by the metacercaria of *C. marginatum* because we have seen natural infections, and some in the catfish industry have been concerned about the esthetic appearance, suitability for the market place and health of individuals infected with even one or few of these yellow grubs (e.g., Mitchell 2002), even though others such as MacMillan (1985) considered infections not to represent a health problem to catfish. Numerous specimens of the catfish as well as the bluegill revealed no infection resulting from exposure to large number of cercaria of what we consider *C. attenuatum*, but *G. affinis* became heavily infected. As also indicated, the reason for confusion about the pathogenicity of the clinostomes to the catfish among biologists and pond managers is because there is more than one species of the strigeid related to the diplostomoids. We are presently investigating material of what we believe to be two or three different species in the Southeast to assess their identity.

Industry-management techniques

According to the US Department of Agriculture (USDA), about 96% of the catfish sales in the US catfish industry are presently pond-reared *Ictalurus punctatus* from Mississippi, Louisiana, Arkansas and Alabama in that order of importance. Mississippi accounted for 82% of the processed catfish in 1995 (Mott and Brunson 1997). The economic value of sales has been decreasing recently as evidenced by 2002-sales of \$410 million being 8% less than in 2001, even though increasing slightly in 2003 (according to the USDA, National Agricultural Statistics Service [NASS]—<http://www.usda.gov/nass>). A major part of that decrease is a result of decreasing prices (Fig. 17). In spite of the decreasing prices, poor marketing efforts, low chicken prices, questionable cooperation within the industry, disease with an emphasis on digenean infections, predation by birds, foreign imports and other factors, the quantity of reported processed farm-raised catfish during the same period has increased steadily from an annual production of 200 million kg in 1994 to 300 million kg in 2003 shown as monthly values in Fig. 18. The increase provides more potential digenean hosts.

Several means exist to manage the digenean infections on the farms. Even though *Pelecanus erythrorhynchos* and at least two species of cormorants feed heavily on catfish as well as disseminate and disperse

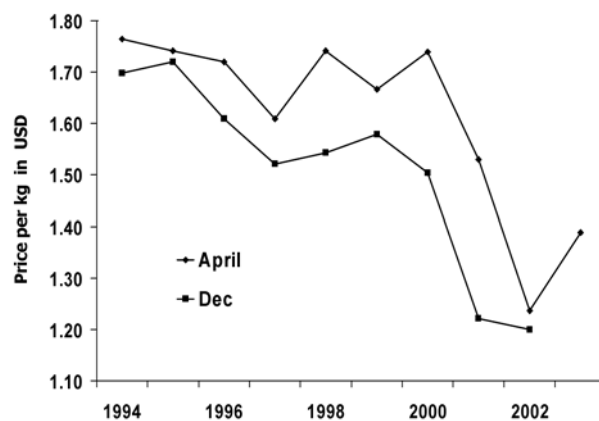


Fig. 17. Recent price per kilogram of farm-raised *Ictalurus punctatus* in southern USA paid to producers by processors in April and December, two months usually exhibiting high and low values each year. Values released by the US National Agricultural Statistics Service (NASS).

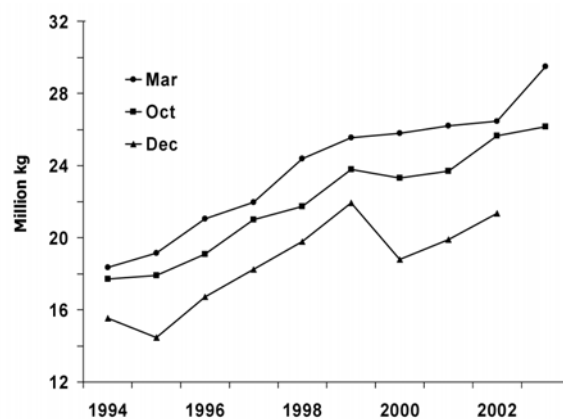


Fig. 18. Quantity of farm-raised *Ictalurus punctatus* in millions of kilograms processed by major US processors in March, October, and December, months usually exhibiting a major peak, secondary peak, and low amount, respectively. Values released by NASS.

four digeneans to various ponds (Fig. 1), controlling the birds is not realistic for controlling the infections. In recent years, the number of *P. erythrorhynchos* (see King and Werner 2001, King and Michot 2002) and cormorants, especially *Phalacrocorax auritus* (see King et al. 1995, Glahn and Dorr 2002), have increased considerably in Louisiana and Mississippi. This increase results from a complexity of factors. The 1973 ban on the nearly three decade application of the pesticide DDT in the USA in 1973 reduced toxic levels of the compound and its byproducts, allowed egg shells to regain their previous strength and, in turn, resulted in relatively high chick survival. That increase in birds

coincided with an increase in catfish production and catfish farms, and, since the migratory routes of both birds extend from at least the Lower Mississippi Valley to northern Great Plains and beyond (Johnsgard 1993, King et al. 1995, King 1997), they have a readily available source of food. They especially like to feed on catfish before they make their northerly migration from their wintering grounds to their breeding grounds (King et al. 1995, King and Michot 2002). Both birds consume a significant number of catfish per day. Less important but still significant predators are wading birds. Glahn et al. (1999) observed the heron *Ardea herodias* and egret *Ardea alba* in northwest Mississippi and found an average of 78 herons and 56 egrets on an average 127-ha farm. The egret ate small catfish among other dietary items, but the diet of the heron consisted of 44% (by weight) larger (16 cm) fingerlings.

Birds temporarily can be kept from ponds by using a variety of methods (Mott and Brunson 1997), including frightening strategies such as pyrotechnics, visual effigies and reflective objects. Exclusion techniques mechanically prevent access to fish, and these include nets, wires, lines strung at different heights and electric fences. Other control methods used separately or in conjunction with above methods include shaping ponds differently, limiting killing of birds, dogs, such as border collies, complete screen enclosures and altering stocking strategies to add fish at a later period or as larger individuals. Nevertheless, none of those methods used by itself is likely to be cost-effective. Moreover, even though a combination of some of the methods can pre-vent most predation, most do not prevent bird hosts from roosting or flying over the ponds. Those individuals can still defecate infective digenean eggs into the ponds. Also, day-to-day operations are inconvenienced by most methods. The best way to control the infections necessitates eliminating or greatly reducing the snail stocks.

Infected snails in ponds can be significantly reduced in numbers by chemical, physical and biological means. Chemical means seem to be the best means to control the snails. Even though 2.5 ppt NaCl was shown to be near optimal for survival of cercaria *Bolbophorus damnificus* (as *B. confusus*), that level was sufficient to kill the snails and thereby eliminate the cercarial infections. That large amount of necessary salt, however, makes such treatment impractical for all but a small pond. It cost about 7.5 times more than that necessary to stock *Mylopharyngodon piceus* (Richardson) (see Venable et al. 2000). Mitchell (2002) found that spraying a 2-m swath of a pond's shoreline, where most of the snails live, with 589 g of copper sulfate and 59 g of citric acid for each 10 m nearly eliminated *Planorbella trivolvis* and presumably other snails. A followup study of that US Environmental Protection Agency (US EPA)-approved method found that admini-

stration of those compounds should be conducted at water temperatures less than 29°C or they were toxic to the fish (Mitchell and Hobbs 2003). When the copper sulfate without citric acid, a treatment considered by those authors to be less toxic to catfish than the citrated compound, was administered at temperatures above 21°C, it was an effective method (Mitchell and Hobbs 2003).

Care has to be used to treat shorelines with copper before catfish fry are stocked, because these fry, unlike fry of many fishes, do not try to avoid toxic levels of copper (Mitchell 2002). However, ponds are typically stocked with the copper-tolerant fingerlings and such stocking as well as harvesting of adults can occur optimally up to three times per year, about the same number of times the copper is added. According to Andrew Mitchell (USDA, ARS, pers. comm.), copper is relatively safe treatment as long as the alkalinity of the pond is at least 150 mg/l as CaCO₃, the ponds are 2 ha large or more, there is no wind when administered and the temperature is above 21°C. This amount of copper relates to considerably less than routinely used for algal control. Copper does not accumulate in catfish flesh. Temperature by itself also has an effect on infections since a decrease from 25 to 15°C inhibits nearly all cercarial release, a reversible condition (Terhune et al. 2002). Snails and infections can survive 10°C for at least 14 days. Consequently, acquisition of new infections is seasonal. Copper is also much more practical than using the US EPA registered herbicide alkyimine salt of endothall (Venable et al. 2000). It has a much greater margin of safety between the toxicity to snails and to catfish and has a much lower cost.

Biological control has been achieved experimentally using triploid carps. *Ctenopharyngodon idella* Valenciennes from Southeast Asia has been used in ponds since 1963 and because of that has escaped into the wild in 48 of the 50 states. Not all individuals are triploid (Fuller et al. 1999, US Fish and Wildlife Service [USFWS], <http://www.fcsc.usgs.gov>). It rids ponds of the infectious digeneans by eliminating the vegetation and, secondarily, the snails. Another carp from eastern Asia, *M. piceus*, introduced into the USA accidentally in the 1970s and intentionally in the 1980s by fish farmers for control of the yellow grub (*C. marginatum*) and as a food fish, favours snails as its primary diet (Fuller et al. 1999, USFWS, <http://www.fcsc.usgs.gov>). At a level of 40 17-cm individuals/ha, it controlled the snail *P. trivolvis* in catfish ponds (Venable et al. 2000). However, that fish grows rapidly and can also be either directly or indirectly harmful to other occupants of the ponds, especially after eating most of the snails. It has not been reported to have escaped into the wild except into Missouri, where it might not be established (Nico and Williams 1996, Fuller et al. 1999), and there is much concern by both the USFWS and US EPA as to

what it would do to the wild invertebrate stocks if it became established or spread. Both carp species can grow very large and are long lived (Froese and Pauly (2003) listed *C. idella* as up to 45.0 kg, 150 cm and 10–21 years old and *M. piceus* up to 32.0 kg, 122 cm and 14 years old, but unsubstantiated data of much larger specimens of both species occur on the Internet).

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